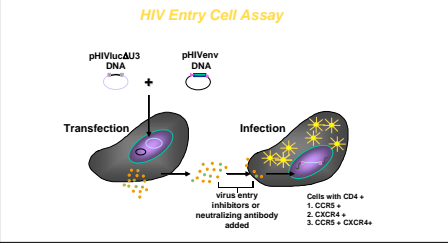
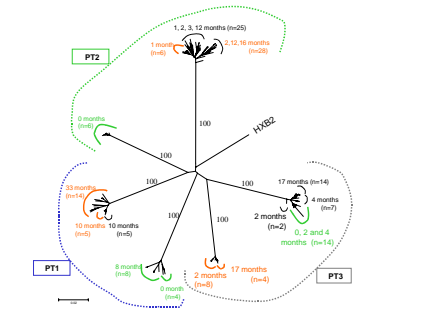


Background: Sequentially expressed dual infections can arise by sequential acquisition of viral variants (superinfection) or through dynamic action of selection pressures in dually infected persons. Recombination of multiple subtypes of HIV-1 is common. Fewer cases of intracade recombination have been reported. Here we report frequent recombination in the envelope gene in patients infected with two, sequentially expressed strains of subtype B HIV-1.

Methods: Plasma samples were identified in three individuals from the UCSF Options cohort before and after apparent superinfection. Antibody neutralization sensitivity and coreceptor tropism were determined using the PhenoSense HIV Entry Assay. Envelope clones were isolated at each time point and their sequences were determined to elucidate the composition of the virus populations



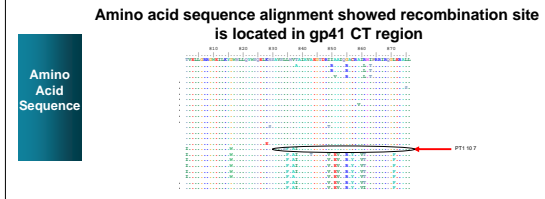
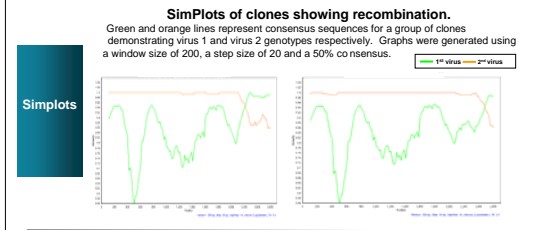
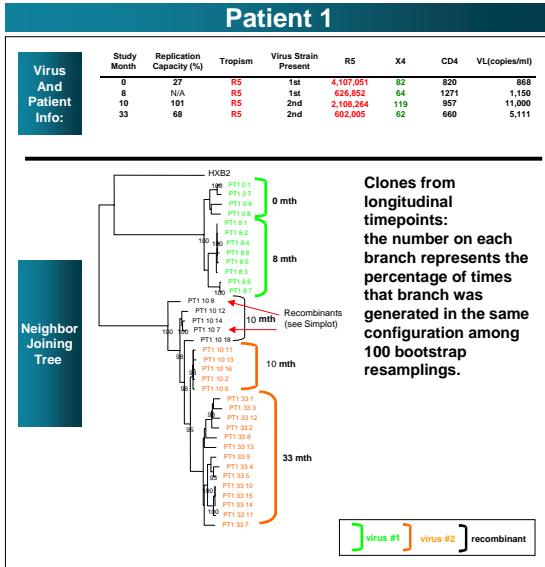
Phylogenetic tree: primary and emergent strains are highly divergent (10% mean genetic distance)



Conclusions: Intrasubtype B recombinant envelope sequences were identified in all 3 cases of apparent superinfection that were studied by clonal analysis. The cytoplasmic tail region was identified as a "hot spot" for recombination in these viruses, which is expected to alter infectivity and fusion phenotypes that may drive viral fitness during transmission and systemic viral spread. The ability of the second strains to overgrow the initial virus population has implications for the development of a broadly protective vaccine.

Acknowledgement

We thank Marie Cardenas for excellent technical assistance.

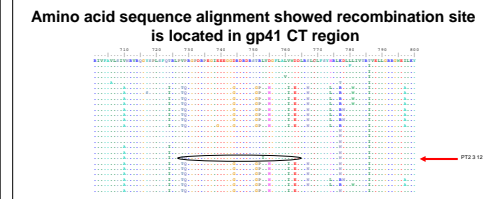
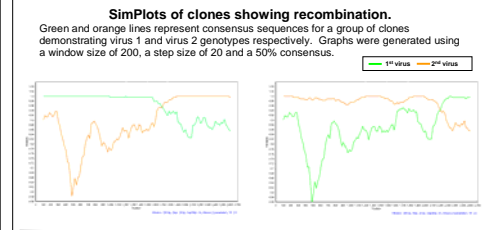
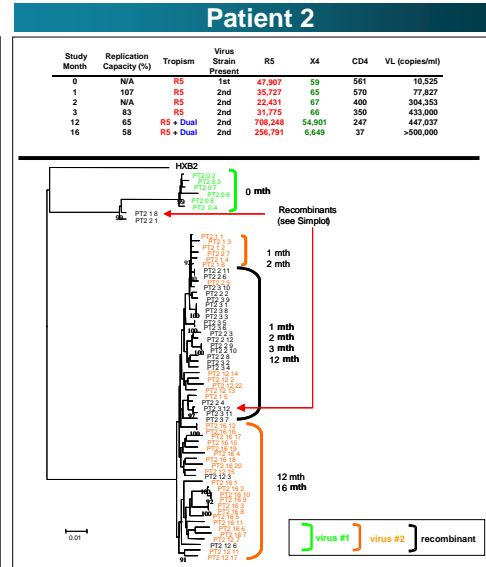


Autologous neutralization before and after emergence of 2nd virus

Virus	0 month plasma	10 month plasma	IC50 ₅₀ Plasmic: N50
OP-480	<20	<20	118
OP-480	10 month plasma	<20	82
Control	<20	<20	39
Control	JNCSF	<20	60
Control	NL43	131	287

Summary Patient 1

- The first virus strain was replaced by the second virus strain or a recombinant of the two.
- Five of 10 clones in the first time point after emergence of the second virus are recombinants. Four recombination crossover points fall in the cytoplasmic tail region of gp41 and one in the transmembrane region of gp41. No recombinants are found in the second time point after emergence of the second virus.
- The second virus strain has higher pol replicative capacity and is less sensitive to autologous neutralizing antisera. Either or both factors may be important in the overgrowth of the first virus by the second.



Autologous neutralization before and after emergence of 2nd virus

Virus	0 month plasma	12 month plasma	IC50 ₅₀ Plasmic: N50
OP-790	<20	<20	78
OP-790	12 month plasma	<20	17
Control	<20	<20	18
Control	JNCSF	<20	372
Control	NL43	<20	65

Summary Patient 2

- The first virus strain was replaced by the second virus strain or recombinant after the appearance of the second strain.
- The recombinants are found at all time points after emergence of 2nd strain.
- The second virus strain switched tropism from R5 to dual 11 months after appearance.
- One of seven clones in the first time point after appearance of 2nd virus, 10 of 12 clones in the second time point, 12 of 12 clones in the third time point, and two of 10 clones in the fourth time point are recombinants. Their recombination sites fall in CT, signal peptide, or gp120/gp41 cleavage site region.
- Both viruses are insensitive to autologous neutralization antibody.

